Comparative Genomic Hybridization: A New Approach to Screening for Intrauterine Complete or Mosaic Aneuploidy

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In the practice of clinical genetics chromosomal aneuploidy in both mosaic and nonmosaic forms has long been recognized as a cause of abnormal prenatal and postnatal development. Traditionally, cytogenetic analysis of cultured lymphocytes has been used as a standard test for detection of constitutional aneuploidies. As lymphocytes represent only one lineage, chromosomal mosaicism expressed in other tissues often remains undetected. The purpose of this study was to assess the utilization of molecular cytogenetic analysis for detection of chromosomal aneuploidy in placental tissues. Using placentas from 100 pregnancies with viable nonmalformed livebirths, both trophoblast and chorionic stroma were analyzed using comparative genomic hybridization (CGH). In all cases with an indication of chromosomal imbalance by CGH, fluorescence in situ hybridization (FISH) analysis was performed to confirm the presence of aneuploidy. To differentiate between constitutional aneuploidy and confined placental mosaicism (CPM), amniotic membrane was analyzed by CGH and FISH techniques. Our results demonstrated five placentas with CPM for chromosomes 2, 4, 12, 13, and 18, respectively, and two constitutional nonmosaic aneuploidies (47,XXX and 47,XXY). Molecular cytogenetic studies of human placental tissues enables easy analysis of both embryonic (amnion) and extraembryonic (chorion) cell lineages. Detection at birth of chromosomal defects affecting intrauterine placental and fetal development is important because these chromosomal defects may continue to have an influence on postnatal development. Am. J. Med. Genet. 92: 281–284, 2000.

KEY WORDS: **intrauterine chromosomal mosaicism; CGH of placental tissue; extraembryonic tissues; confined placental mosaicism**

INTRODUCTION

Chromosomal aneuploidy is defined as the presence of an extra or absent chromosome in a diploid complement. It is reported in approximately 0.3% of liveborn infants using cord blood for cytogenetic analysis. Chromosomal aneuploidy occurs in mosaic and nonmosaic forms [Hassold et al., 1993]. Generalized chromosomal mosaicism originates from an early postzygotic mutational event, with both embryonic and extraembryonic tissues showing normal and aneuploid cell lines. In contrast, confined forms of chromosomal mosaicism affect only some embryonic or extraembryonic tissues [Kalousek, 1993] and are less readily identified as the standard postnatal test when a cytogenetic defect is suspected is cytogenetic analysis of cultured peripheral blood.

Cytogenetic studies of the human placenta established the existence of a clinically significant condition known as confined placental mosaicism (CPM). CPM is defined as a tissue-specific mosaicism involving a cytogenetic abnormality, most often trisomy, confined to the placenta and absent in the fetus. CPM is detected in approximately 1–2% of viable pregnancies studied by chorionic villus sampling at 10–12 weeks of gesta-

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tion. Categorized according to the specific placental cell lineage exhibiting the abnormal cell line, three types of CPM have been described. Placental mosaicism can be confined either to trophoblast (Type I), chorionic stroma (Type II), or both cell lineages (Type III) [for review, see Lestou and Kalousek, 1998].

The reliability of traditional cytogenetic studies to identify chromosomal defects in the extraembryonic placental lineages is limited, as trophoblast is not easily grown in culture and cultural artifacts are common in long-term cultures of chorionic stroma [Kalousek et al., 1998]. The limitations associated with traditional cytogenetic analysis were overcome by the development of molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). A molecular cytogenetic approach represents the most effective means available for postnatal investigations of placentas at the present time [Kalousek et al., 1998]. In this study, 100 placentas from pregnancies with viable nonmalformed livebirths were analyzed by CGH. Using a liberal interpretation criterion, the CGH results indicated possible aneuploidy in 21 placentas and follow-up FISH analysis confirmed the CGH findings in seven cases. Our results demonstrate both the sensitivity and limitations of this molecular cytogenetic approach and highlight the value of cytogenetic analysis of placental tissues.

MATERIALS AND METHODS

Placental Tissue Collection and Analysis

Following delivery of a viable and nonmalformed infant, samples of chorionic villi and amniotic membrane were taken from 100 placentas. Chorionic villus samples were enzymatically separated into trophoblast and chorionic stroma as described by Henderson et al. [1996]. DNA was extracted from a portion of each cell suspension for CGH analysis using standard protocols. The remaining cell suspensions were processed for FISH analysis as needed. In all cases with aneuploidy detected in chorionic villi, amniotic membrane was analyzed by CGH and FISH in the same manner.

CGH and FISH

The CGH methodology used for this study, including preparation of metaphase chromosomes, DNA labeling, and hybridization conditions, was described in detail by Lestou et al. [1999]. Six high-quality metaphase spreads were analyzed from each hybridization. We considered any shifts in the average red-to-green ratio value (plus two standard deviations) above 1.0 as an indication of increased copy number and any shifts below 1.0 as an indication of reduced copy number. For all CGH-positive cases, FISH analysis was performed for the indicated chromosome. Heterochromatic regions near the centromeres of chromosomes 1, 9, 16, the q arm of the Y chromosome, and the satellite regions of the acrocentric chromosomes were not considered in the CGH analysis, as these regions are polymorphic and are suppressed with Cot-1 DNA.

RESULTS

Among the 100 analyzed placentas, 21 demonstrated aneuploidy in trophoblast, stroma, or both using our interpretation criteria for CGH profiles. In seven cases, the aneuploidies identified by CGH were confirmed with FISH (Table I). Two cases demonstrated nonmosaic constitutional aneuploidy for chromosome X. Cases 1 (female infant) and 2 (male infant) demonstrated gains for chromosome X in all three cell lineages (trophoblast, chorionic stroma, and amnion), thus identifying constitutional aneuploidy for this sex chromosome (Fig. 1). FISH analysis demonstrated levels of X chromosome trisomy greater than 74% for the 47,XXX case and levels of X chromosome disomy greater than 92% for the XXY case (Table I). The remaining five cases showed no aneuploidy in amniotic membrane by CGH, thus demonstrating CPM (Fig. 2). FISH follow-up experiments confirmed the CGH results and defined levels of trisomy for the different chromosomes between 11% and 78% (Table I). In detail, Case 3 (male infant) showed CPM Type I for chromosome 13, i.e., mosaicism affecting only the trophoblast. Cases 4 (female infant) and 5 (male infant) demonstrated CPM Type II for chromosomes 2 and 12, respectively, i.e., mosaicism affecting only the chorionic stroma. Finally, Cases 6 (male infant) and 7 (male infant) demonstrated CPM Type III for chromosomes 4 and 18, respectively, i.e., mosaicism affecting both trophoblast and chorionic stroma. To rule out the possibility of a false-positive result, CGH analysis was repeated in cases which demonstrated low levels of trisomy CPM (1–4), and the presence of trisomy was demonstrated again in all cases.

The findings from the other 14 CGH-positive cases were not confirmed by FISH analysis (Table II). FISH

TABLE I. Cytogenetic Analysis of the Seven Aneuploid Cases Among 100 Placentas*

	CGH			FISH % trisomy			
	Tropho	Stroma	Amnio	Tropho	Stroma	Amnion	Aneuploidy
Case 1	XXX	XXX	XXX	74	88	N/A	Constitutional nonmosaic
Case 2	XXY	XXY	XXY	92	99	N/A	Constitutional nonmosaic
Case 3	+13	d	d	13	d	d	CPM type I
Case 4	d	+2	d	d	21	d	CPM type II
Case 5	d	+12	d	d	15	d	CPM type II
Case 6	+4	+4	d	11	22	d	CPM type III
Case 7	+18	+18	d	75	78	d	CPM type III

*d, disomy; N/A, not analyzed; tropho, trophoblast; stroma, chorionic stroma; amnion, amniotic membrane.

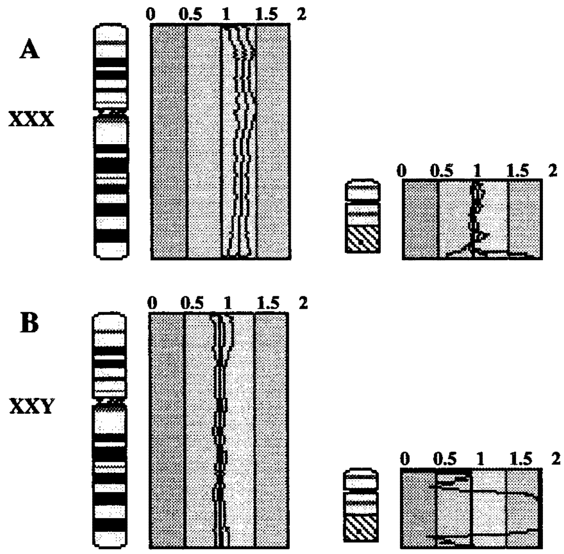

Fig. 1. Composite diagram of average red-to-green fluorescence intensity ratio profiles for XXX and XXY cases as detected in amnion (reference genome 46,XX). **a:** Test DNA XXX. The additional X is detected as gain (1:1.3). Chromosome Y gives DAPI background profile readings (1:1), indicating absence of Y chromosome. **b:** Test DNA XXY. Two X chromosomes are detected (1:1 ratio). Presence of Y chromosome indicated (1:2 ratio).

analysis demonstrated levels of trisomy as low as 1.2% and up to 10%, but below the cut-off values for trisomy for these chromosomes. Ten false-positive cases involved single trisomy for chromosomes 2, 4, 13, 17, 18, 19, and 20. Double trisomy for chromosomes 19/22 and triple trisomy for chromosomes 4/13/18 were each detected twice and not confirmed by either FISH or repeat CGH analysis (Table II). CGH analysis was repeated for seven of the false-positive cases involving chromosomes 4, 13, 1, 19, and 22, and, consistent with the FISH results, none of the initial positive CGH findings recurred.

DISCUSSION

While traditional cytogenetic studies can readily detect constitutional nonmosaic forms of aneuploidy in cord blood [Jacobs and Hassold, 1987], the detection of confined mosaicism is more difficult and requires the utilization of molecular cytogenetic technology [Kalousek et al., 1998]. To make an accurate diagnosis of mosaic or nonmosaic forms of intrauterine chromosomal aneuploidy, both embryonic and extraembryonic cell lineages should be evaluated. These tissues are readily obtained from the placenta at birth. In this study, CGH analysis was used to screen the genetic complement of 100 placentas. In combination with FISH, this technique enabled us to detect and confirm low- and high-level trisomy placental mosaicism in five cases and constitutional nonmosaic aneuploidy in two cases.

The major limitation of CGH alone for the detection of mosaicism is the high rate of false-positive findings. The false-positive results are due to the variability inherent in the CGH technique. This variability is demonstrated by the lack of relationship between the de-

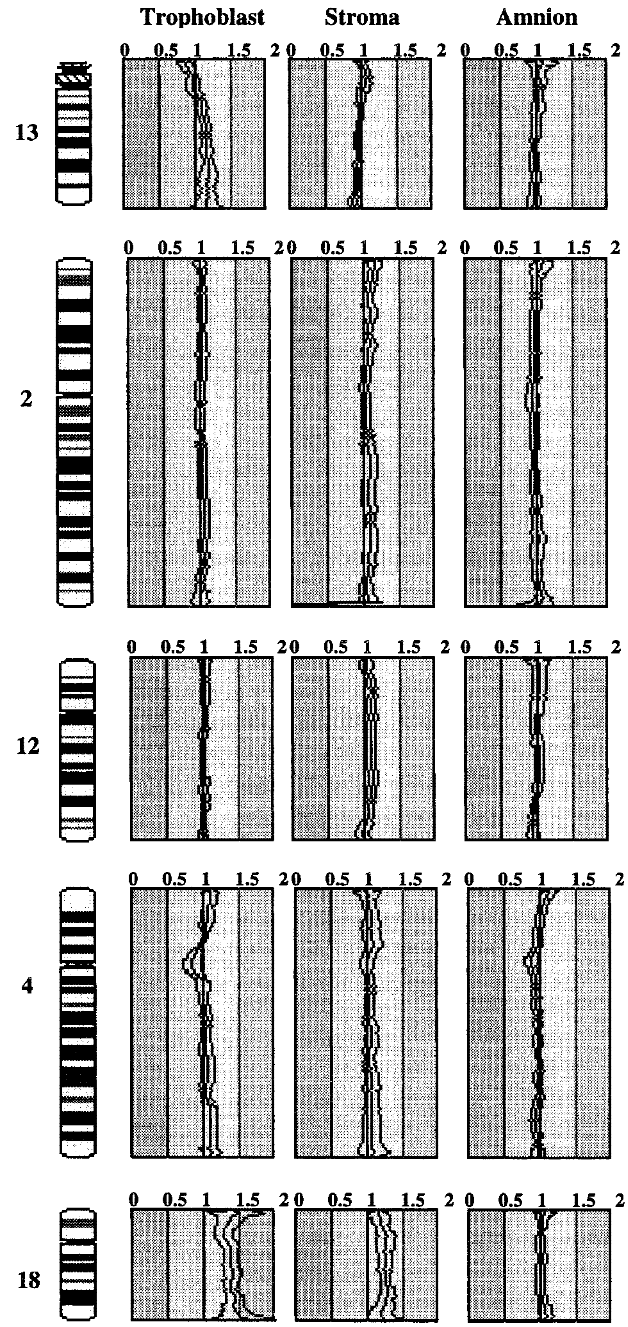

Fig. 2. Composite diagram of average red-to-green fluorescence intensity ratio profiles for CPM cases. CPM Type I gain for chromosome 13 (1:1.25). CPM Type II gain for chromosome 2 (1:1.13) and gain for chromosome 12 (1:1.2). CPM Type III is demonstrated by gain for chromosome 4 (1:1.1 and 1:1.12) and gain for chromosome 18 (1:1.4 and 1:1.3) in trophoblast and stroma, respectively. Amnion is diploid in all cases (1:1 ratio).

gree of shift in the ratio profile and the percentage of aneuploidy as determined by FISH [Lestou et al., 1999]. Our results indicate that by employing a CGH interpretation criterion which detects low-level mosaicism, one will inevitably have false-positive findings. However, using follow-up FISH analysis these can be readily differentiated from true mosaics. Conversely, a more stringent CGH interpretation criterion can be employed which avoids the problem of false-positive

TABLE II. Fourteen False Positive Cases Detected by CGH Among 100 Placentas*

	Chromosome	Affected Tissue	FISH % trisomy	Repeated CGH
Case 1	2	Trophoblast	2	N/A
Case 2	4	Trophoblast	4	N/A
Case 3	13	Stroma	8	d
Case 4	13	Stroma	3	N/A
Case 5	17	Stroma	4	N/A
Case 6	18	Trophoblast	1	N/A
Case 7	19	Trophoblast	6	d
Case 8	19	Trophoblast	10	d
Case 9	19	Stroma	4	N/A
Case 10	20	Trophoblast	3	N/A
Case 11	19/22	Trophoblast/Stroma	3–8	d
Case 12	19/22	Trophoblast/Stroma	2–5	d
Case 13	4/13/18	Trophoblast/Stroma	1–8	d
Case 14	4/13/18	Trophoblast/Stroma	3–6	d

*N/A, not analyzed; d, disomy.

findings but will not detect all cases of mosaicism. Clearly, the interpretation criterion must reflect the aims of the CGH analysis.

In addition, certain "problem chromosomes" (1pter, 16p, 19, and 22) may show deviations from a 1.0 fluorescence intensity ratio in normal diploid cases. This observation has been previously reported to be artifactual [Kallioniemi et al., 1993] and is demonstrated in this study as well. Based on our experience with placental tissues, similar effects may be observed for chromosomes 4, 13, and 18. With experience these artifact patterns can be readily identified and once recognized such profiles can be carefully evaluated, thereby avoiding false-positive diagnosis.

One of the frequently asked questions is, can CGH be widely applied in clinical diagnosis [Warburton, 1999]? A recent study of spontaneous abortions clearly demonstrates that CGH in combination with flow cytometry has a lower failure rate than conventional cytogenetic analysis and provides more accurate cytogenetic results by avoiding maternal contamination and tissue culture artifacts [Lomax et al., 1999]. CGH technique has been shown to be a useful adjunct tool for determining the origin of extrachromosomal material, even in cases where abnormalities are judged to be subtle [Breen et al., 1999]. And further, our results demonstrate that CGH in combination with FISH is a time- and cost-effective approach for screening term placentas for aneuploidy.

Molecular cytogenetic analysis of placental tissues may be a useful diagnostic tool for pediatricians and medical geneticists in the assessment of a newborn infant with an unrecognized pattern of anomalies with or without growth retardation. Retrospective molecular cytogenetic analysis of frozen placental tissues may also be highly informative in infants and children with idiopathic abnormal development. The benefits of CGH analysis of the placenta include the detection of CPM, which is the result of abnormal cell division in early development. CPM has been reported in association with clinically significant intrauterine growth restriction (IUGR) and in some cases fetal uniparental disomy for the chromosomal pair which is trisomic in the placenta [Kalousek, 1993]. Detection of placental aneuploidy could also indicate cryptic generalized mosaicism responsible for abnormal development in the child. Furthermore, in pregnancies with CPM associated with an IUGR infant it is important to acknowledge the value of the recent findings supporting a concept of programming the fetus by undernutrition in utero [Barker, 1997]. In fetal life, the tissues and organs of the body go through so-called "critical" periods of development coinciding with periods of rapid cell division. Undernutrition related to placental mosaicism may therefore permanently change the "program" of the organs and body influencing the distribution of cell types, hormonal feedback, metabolic activity, and organ structure. Retrospective molecular cytogenetic analysis of frozen placental tissues collected routinely at delivery and detection of CPM may represent an important diagnostic tool for pediatric practitioners and medical geneticists in understanding of an abnormal postnatal development.

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